US 20060233886A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2006/0233886 A1**

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(54) **ANTIMICROBIAL COMPOSITION AND METHOD FOR MAKING SAME**

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(21) Appl. No.: **11/381,269**

(22) Filed: **May 2, 2006**

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/095,329, filed on Mar. 31, 2005.

Publication Classification

(51) **Int. Cl.**
A61K 33/42 (2006.01)
A61K 33/40 (2006.01)
A61K 31/19 (2006.01)

(52) **U.S. Cl.** **424/601**; 424/616; 514/557

(57) **ABSTRACT**

Anti-microbial Formulations and methods of their use and production are disclosed. The Formulations of the present invention are effective as broad spectrum anti-bacterial agents with efficacy against both Gram-negative and Gram-positive bacteria, as anti-viral agents with efficacy against both enveloped and non-enveloped viruses, anti fungal agents and anti spore forming agents. The present invention includes anti-microbial Formulations that include at least one surfactant, optionally at least one acid, at least one non-cationic anti-microbial agent, and optionally water. The anti-microbial Formulations of the present invention may additionally contain an organic salt. The organic salt may be a salt of the same acid that is used in the Formulation or a salt of a different acid.

ANTIMICROBIAL COMPOSITION AND METHOD FOR MAKING SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of application Ser. No. 11/095,329, filed Mar. 31, 2005, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to chemical Formulations that possess anti-bacterial, antiviral and anti-fungal properties and methods of making and using those Formulations.

[0004] 2. Description of the Background

[0005] Bacteria, viruses and fungi, e.g., mold, are a major source of disease and contamination throughout modern society. The need to control the growth of these microorganisms is paramount for maintaining public health as well as reducing costly commercial and industrial contamination. In the case of bacteria, infections and contaminations are effected by both Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative (e.g., *Escherichia coli*) bacteria. Many anti-bacterial agents are limited in their efficacy to only one of those two classes of bacteria. Moreover, known anti-viral agents are often effective only against enveloped or non-enveloped viruses, but not both.

[0006] While numerous anti-microbial agents exist, many have the further limitation that they cannot be compounded into Formulations for extended transport over concerns regarding degradation of stability and/or efficacy. Thus, there has been a long standing need for stable and efficacious anti-microbial Formulations that possess broad spectrum activity.

Definitions

[0007] As used herein, unless otherwise indicated, the following terms mean:

[0008] Microbe(s) includes bacteria, viruses, fungi, mold, and spore-forming bacteria;

[0009] Inhibit means substantially stopping the growth of a Microbe(s);

[0010] Kill means substantially causing the death of a Microbe(s);

[0011] Anti-microbial agent(s) means a compound or composition which in liquid, solid or gaseous form, Inhibits or Kills Microbes.

SUMMARY OF THE INVENTION

[0012] The present invention generally relates to anti-microbial Formulations and methods of their use and production. The Formulations of the present invention are effective as broad spectrum anti-bacterial agents with efficacy against both Gram-negative and Gram-positive bacteria, as anti-viral agents with efficacy against both enveloped and non-enveloped viruses, anti fungal agents and anti spore forming agents. The present invention includes anti-microbial Formulations that include at least one surfactant, optionally at least one acid, at least one non-cationic anti-microbial agent, and optionally water. The anti-microbial Formulations of the present invention may additionally contain an organic salt. The organic salt may be a salt of the same acid that is used in the Formulation or a salt of a different acid. More particularly, we have discovered an anti-microbial composition comprising:

[0013] at least one surfactant present in a concentration from about 3% to about 95% by weight;

[0014] an acid present in a concentration of up to about 20% by weight;

[0015] at least one non-cationic anti-microbial agent and

[0016] the balance water,

[0017] wherein the amount of anti-microbial agent is effective to produce a Zone of Inhibition of from 15 to 150 mm and for the composition to exhibit Plate Cidality against each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 and exhibits a negative cytopathic effect (is CPE negative) at dilutions up to 1:2000.

[0018] In one embodiment, we have discovered that the inventive efficacious antimicrobial Formulations can be prepared when a solid antimicrobial agent is introduced into the Formulation above its melting point and a minimum level of surfactant is present in the Formulation to provide a clear and homogeneous Formulation both initially and upon sequential dilution with water. A straight forward and easy to use procedure is described to determine the required minimum level of surfactant and to determine whether the Formulation has the necessary features and characteristics required for an efficacious antimicrobial Formulation.

[0019] In yet another embodiment, a solid antimicrobial is combined with another liquid antimicrobial agent that can dissolve or liquefy the solid antimicrobial agent. In this embodiment, it is not necessary to employ heat to melt the solid antimicrobial agent. However, in this embodiment, a clear, homogeneous Formulation is obtained which on sequential dilution exhibits a high degree of antimicrobial efficacy. In particular, if a peroxide is used as a part of the antimicrobial agent, then it is possible to dissolve the solid antimicrobial agent in water containing the peroxide at a temperature below its melting point and still achieve the characteristic antimicrobial properties of the inventive composition.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The present invention broadly relates to anti-microbial compositions comprising at least one surfactant, optionally at least one acid, at least one non-cationic anti-microbial agent, and optionally water. The anti-microbial Formulations of the present invention may additionally contain an organic salt. The organic salt may be a salt of the same acid that is used in the Formulation or a salt of a different acid.

[0021] The Formulations of the present invention includes a surfactant. The surfactant used in the present Formulation may be amphoteric, cationic, nonionic or anionic. The most

preferred surfactants are amphoteric members of the following classes of chemical compounds: alkylamine oxides, alkyamidopropyl amine oxides, alkyl betaines, alkyamidopropyl betaines, and sultaines. Specific examples of alkyl amine oxides that may be used in the present invention include octyl amine oxide, decyl amine oxide, lauryl amine oxide, iso-dodecyl amine oxide, myristyl amine oxide, cetyl amine oxide, oleamine oxide, stearyl amine oxide, and palmitamine oxide. Specific examples of alkylamidopropyl amine oxides that may be used in the present invention include laurylamidopropyl amine oxide, cocamidopropyl amine oxide, stearamidopropyl amine oxide, germamidopropyl amine oxide. Specific examples of alkyl betaines that may be used in the present invention include octyl betaine, lauryl betaine, cocobetaine, cetyl betaine, oleyl betaine, and tallow dihydroxylethyl glycinate. Specific examples of alkylamidopropyl betaines that may be used in the present invention include caprylamidopropyl betaine, capramidopropyl betaine, lauamidopropyl betaine, cocamidopropyl betaine, isostearamidopropyl betaine, wheatgermamidopropyl betaine, and coco/sunfloweramidopropyl betaine. Specific examples of sultaines that may be used in the present invention include cocamidopropyl hydroxysultaine and lauryl hydroxysultaine. The examples provided above are not an exhaustive list of the surfactants that may be used in the present invention. One of skill in the art will recognize additional members and variations within the various categories listed above. Such additional compounds are considered to be within the scope of the present invention.

[0022] A single surfactant of the types listed above may be used in the Formulations. Alternatively, Formulations that include multiple surfactants are also considered as within the scope of the present invention.

[0023] Particularly preferred surfactants that may be used in the present invention include alkali metal salts of alkylamphoacetates, dialkali metal salts of alkylamphoacetates, alkali metal salts of alkylamphopropionates, dialkali metal salts of alkylamphopropionates, alkanolamides, disodium alkyl sulfosuccinates, monsodium diakyl sulfosuccinates, disodium alkylethoxy sulfosuccinates, sodium ethoxydimethicone sulfosuccinates, disodium propoxydimethicone sulfosuccinates, disodium alkylamido MEA sulfosuccinates, disodium alkylamido MPA sulfosuccinates, sodium alkylamidopropoxy sulfosuccinates, alkylamidopropylmorpholine lactate, and ethylene oxide/propylene oxide diblock and triblock surfactants.

[0024] The antimicrobial agents that are preferably employed in the present invention are non-cationic antimicrobial agents. The non-cationic antimicrobial agents may be selected from the groups of phenolics, halogenated phenolics, halogenated diphenyl ethers, halogenated carbonilides, water soluble- or water insoluble peroxy oxidizing agents (e.g. peroxides, peresters, peracids, persulfates), or mixtures thereof.

[0025] More specifically, typical antimicrobials in this include:

[0026] Phenolics:

[0027] Phenol—melting point 43° C.

[0028] Xylenol—melting point 75° C.

[0029] 2-nitrophenol—melting point 45° C.

[0030] 2-phenyl phenol—melting point 57-59° C.

[0031] Halogenated Phenolics:

[0032] 2,3-dichlorophenol—melting point 55-57° C.

[0033] 2,4-dichlorophenol—melting point 42-43° C.

[0034] Halogenated Diphenyl Ethers

[0035] Triclosan—melting point 56-58° C.

[0036] Peroxides: 4

[0037] Dicumyl peroxide—melting point 39-41° C.

[0038] Lauroyl peroxide—melting point 53-57° C.

[0039] t-butylhydroperoxide and hydrogen peroxide

[0040] Peracids:

[0041] Peracetic acid—melting point 56° C.

[0042] Peresters:

[0043] t-butyl peracetate—melting point 38° C.

[0044] t-butyl perbenzoate—melting point 93° C.

[0045] Persulfates:

[0046] Sodium persulfate—melting point 100° C.

[0047] The Formulations of the present invention may also include a wetting agent at a concentration of up to about 3%, by weight. For example, commercially available wetting agents, such as, Fluorocarbon and silicone based wetting agents are particularly effective.

[0048] The Formulations of the present invention containing the various components indicated hereinabove may be in the form of a liquid, viscous liquid, liquid, e.g., a liquid soap, a pasty mixture, e.g., a heavy-duty soap used by mechanics, or a semi-solid or solid, e.g., a bar of soap. This depends on the solids content of the Formulation and the present invention contemplates all of such forms. However, the form of the inventive composition does not affect its antimicrobial properties. The inventive formulation, in such forms, may have components in the following amounts (All percent weights herein are based on the total weight of the Formulation):

Formulation	Range		Preferred Range		Most Preferred Range	
Component (% by weight)	Liquid[1] Form	Solid[2] Form	Liquid Form	Solid Form	Liquid Form	Solid Form
Solids % by weight	1–50	50–98	10–50	60–98	15–50	50–95
Surfactant	0.2–98		0.4–50		5–30	
First Solid Antimicrobial Agent	0.0–25		0.5–18		0.1–8.0	
Second Liquid Antimicrobial Agent	0–30		0–20		0–15	
Acid	0–20		0–12		1–10	

-continued

Formulation	Range		Preferred Range		Most Preferred Range	
Component (% by weight)	Liquid[1] Form	Solid[2] Form	Liquid Form	Solid Form	Liquid Form	Solid Form
Acid salt	0–7		0–5		0–3	
ppm Transition Metal Ion	0.0–a catalytica⑦ effective amount		5–500		10–500	

[1]Solid at room temperature
[2]Liquid at room temperature
[3]Only needed if a peroxy antimicrobial agent is present. Includes metal ions such as iron, copper, manganese, chromium, cobalt, nickel, vanadium, tungsten, molybdenum and combinations thereof.
⑦ indicates text missing or illegible when filed

[0049] Occasionally peroxy compounds such as hydrogen peroxide, t-butyl hydroperoxide, benzoyl peroxide and peracetic acid are inactive or exhibit retarded reactions in the absence of catalytic amounts of certain transition metal ions. For example, in radical emulsion polymerization of unsaturated acrylic monomers using hydrogen peroxide or t-butyl hydroperoxide a small amount of aqueous iron sulfate solution is added to insure the peroxy compounds are active and polymerization initiates as soon as the peroxy compound is added to the reactor. In the preparation of our antimicrobial formulations, a catalytically effective amount of a transition metal ion, such as, iron sulfate may be included to achieve the desired antimicrobial effect. Besides iron, copper, manganese, chromium, cobalt, nickel, vanadium, tungsten or molybdenum ions or combinations may be used.

[0050] Peroxy compounds such as hydrogen peroxide or peracetic acid are especially useful to further enhance the efficacy of antimicrobial formulations against difficult to control microorganisms, especially spore forming microorganisms. However, these types of peroxy compounds may exhibit instability in the presence of antagonistic compounds, such as surfactants, which have chemical functionality that can react with the peroxy compound. For example, surfactants based upon natural feedstocks like coconut and palm oils, such as cocobetaines, contain carbon double bonds and conjugated unsaturation which peracetic acid will react with. Often this reactivity or instability of peracetic acid is evident by degassing of the formulation and pressure build up in storage containers. This instability of peracetic acid in the presence of other functional organic materials may require the peracetic acid formulation be used immediately after preparation because of its short active life span or must be mixed at point of use. The mixing at point of use presents several handling and safety issues in view of peracetic acid's oxidizing ability, corrosiveness and toxicity. Therefore there is a need to develop a very potent and stable peroxy containing antimicrobial formulation that is easy to apply and use. We have developed a process which results in highly efficacious and stable peroxy antimicrobial formulations. These new formulations can contain mixtures of non cationic and nonperoxy antimicrobial agents, such as Triclosan, with one or more peroxy antimicrobial agents such as hydrogen peroxide, t-butyl hydroperoxide, peracetic acid, and the like.

[0051] The stable peroxy formulation are prepared by initially adding from about 15 to 50% by weight of stabilized hydrogen peroxide solution to a surfactant, an optional acid, and water. The mixture is then heated at approximately 60° C. for about 8 to 12 minutes. Thereafter, a noncationic antimicrobial agent is added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at about 60-63° C. for 10 minutes. The formulation is cooled to room temperature. The peroxide component, for example, hydrogen peroxide, t-butyl hydroperoxide, peracetic acid, and the like may then be added and the mixture is stirred from one to five minutes to form a clear and homogeneous solution which will not degas. Further dilution of the formulation with stabilized hydrogen peroxide solution or an aqueous solution of iron sulfate provides additional stability or efficacy. The formulation can also be diluted with an aqueous solution of iron sulfate or water.

[0052] The Formulations of the present invention may include acid at a concentration of up to about 20%, by weight. The acid may be an organic acid such as citric acid or an inorganic acid such as phosphoric acid, a mono or multi salt of the acids, e.g., sodium, potassium, ammonium, lithium, and the like. Such acids and acid salts function as a buffer for the Formulation. The balance of the Formulations of the present invention is preferably water in a percentage by weight that is determined by the percentages of the various components that are specified above. The percent solids or percent nonvolatiles components in the Formulation may be up to 98%.

[0053] The Formulations of the present invention may be prepared by two different methods depending on the form of the antimicrobial agent, i.e., whether it is a solid or liquid at ambient temperatures. In a first preparative method wherein the antimicrobial agent is solid, a container may be charged with water, surfactant, and optionally, an organic acid for pH control. The contents of the container are stirred and heated to the temperature of the melting point of the solid antimicrobial agent to be used. A wetting agent may be added at that step in the process. The solid non-cationic anti-microbial agent is added to the heated mixture with rapid stirring. The solid anti-microbial agent, should be allowed to dissolve fully. After the anti-microbial agent is fully dissolved, the solution is stirred at this temperature for about 20 minutes.

[0054] In an alternative embodiment, if the surfactant is also a solid, the solid antimicrobial agent and solid surfactant are added to the container without water or acid and the container heated to convert the a antimicrobial agent to a liquid which then dissolves the surfactant. The mixture is then stirred to fully intermix the components. Water can then be added along with acid as required for pH adjustment, and the mixture cooled to ambient temperature. The pH is in a range from about 2.2 to 8.3.

[0055] In the second preparative method, a first solid antimicrobial agent which is a solid at ambient temperature is admixed with a second antimicrobial agent which is a liquid at ambient temperature to dissolve the solid antimicrobial in the liquid agent. Alternatively, if the liquid antimicrobial agent does not fully dissolve the solid agent, water or a mixture of water and surfactant may be added to complete the dissolution.

[0056] The antimicrobial agent may be formulated as high solids products, e.g. 95% or more solids for example in the form of a soap bar, which when moistened with water exert

the antimicrobial effect. Alternatively, it can be formulated in aqueous dilutions of varying proportions depending on the particular end use contemplated.

[0057] An important property of the present invention is that the Formulations produced as described above when mixed with water produce homogeneous, clear solutions, and display superior stability making them particularly useful for a wide variety of antimicrobial applications. Inasmuch as the microbe to be inhibited or killed are present in aqueous phase, the homogeneity of the resulting solutions in critical to producing the high antimicrobial efficacy of the present invention. The resulting diluted or full strength Formulations may be tested for anti-microbial activity in the following manner.

[0058] The anti-microbial activity of the Formulations of the present invention is expressed herein in three ways:

[0059] 1. Zone of Inhibition

[0060] The Zone of Inhibition of the inventive formulation is determined as follows:

[0061] disks impregnated with known concentrations of antimicrobial Formulations are placed onto the surface of Mueller-Hinton Agar 150 millimeter plates that have been freshly seeded with a known quantity of bacteria (varying from 10/5 to 10/8 CFU/ml). a variety of bacteria can be challenged in this manner in order to determine the spectrum of activity of the antimicrobial agent. The standard inoculum is a 0.5 McFarland standard turbidity, which is approximately 1.5×10/8 CFU/ml. The surface of the agar is swabbed in three directions to ensure an even and complete distribution of inoculum over the entire plate. Within 15 minutes of inoculation, the antimicrobial agent disks are applied and the plates are inverted for incubation to avoid accumulation of moisture on the agar surface that might interfere with the interpretation of the test results. For most organisms, incubation is at 35° C. in air but increased CO_2 is used with certain fastidious bacteria. The dynamics and timing of antimicrobial agent diffusion to establish a concentration gradient coupled with the growth of organisms over an 18- to 24-hour duration is desired to obtain reliable results. Therefore, incubation of disks beyond that allotted time was preferably avoided.

[0062] Using a dark background and a reflected light, the plate is situated so that a ruler or caliper may be used to measure inhibition zone diameters for each antimicrobial agent. Zones are recorded in millimeters (mm) of diameter. Known controls (including media controls) are employed with each run to assure accuracy of techniques employed.

[0063] 2. Plate Cidality

[0064] A loop full of inoculum is taken within the Zone of Inhibition as measured above approximately 2 millimeters from the disk edge after the plates have been incubated for 18-24 hours. The loop is inoculated to a fresh Trypticase Soy Agar plate and then incubated at 35° C. for 18-24 hours before being read. The results were recorded as either "No growth", indicating Plate Cidality or "Growth" indicating no Plate Cidality. Alternatively, the number of colonies was recorded in cases where there was incomplete Plate Cidality.

[0065] 3. Antiviral Activity

[0066] The antiviral activity of the inventive formulation is expressed as a negative or positive CPE. CPE negative means that the Formulation allows no growth of an ATCC culture of Herpes Simplex virus: CPE positive means that the formulation is not effective and allows growth under the test conditions. The test to determine whether the formulation is CPE positive or negative is carried out as follows:

[0067] MRC-5 human embryonic diploid lung fibroblast cells are inoculated with a previously germicide treated ATCC culture of Herpes Simplex virus maintained in Eagle's minimum essential medium. The treatment is with the Formulation to be tested at a 1:3 dilution held at room temperature for 1 hour prior to cell inoculation. Two shell vials are inoculated in this manner and then incubated at 35 C, one for 24 hours and the other for 48 hours. After incubation they are observed for cytopathic effect (CPE) using standard immunofluorescent staining. CPE negative is considered to be an effective kill of HSV. CPE positive is considered to be a failure. Positive and negative controls are run with all experiments.

[0068] As noted above, an important property of the inventive formulation is that when diluted with water it provides a clear homogeneous mixture which, in turn, allows it to be an effective and efficient anti-microbial agent. Table 1 sets forth the ratios and amounts of surfactant and solid antimicrobial agent to achieve a clear homogeneous mixture on sequential aqueous dilution using the first embodiment outlined hereinbefore wherein the surfactant is cocobetaine (low salt version) or a mixture of cocobetaine (low salt version) and lauryl amine oxide and the solid antimicrobial is triclosan (triclosan is 2,4,4'-trichloro-2'-hydroxydiphenyl ether; it is also known as 5-chloro-2-(2,4-dichlorophenoxy)phenol).

TABLE 1

Requirements for Clear Homogeneous Antimicrobial Formulation Both Initially and Upon Sequential Dilution

Formulation No.[4]	Surfactants weight %[5]	Surfactants/ Triclosan Ratio[6] (By Wt.)	Temperature ° C.[7]	Initial Clarity	Sequential Dilution Clarity
22	24% CB-LS/2% LAO	6.9	60	YES	YES
5	25.7% CB-LS/0.9% LAO	5.9	60	YES	YES
1	12% CB-LS/0.4% LAO	5.6	60	YES	YES
2	12% CB-LS/0.4% LAO	5.6	60	YES	YES

TABLE 1-continued

Requirements for Clear Homogeneous Antimicrobial Formulation Both Initially and Upon Sequential Dilution

Formulation No.[4]	Surfactants weight %[5]	Surfactants/ Triclosan Ratio[6] (By Wt.)	Temperature ° C.[7]	Initial Clarity	Sequential Dilution Clarity
45	12% CB-LS/0.4% LAO	5.6	RT	NO	—
23	12% CB-LS	5.6	60	YES	YES
24	25% CB-LS/1.3% LAO	5.2	60	YES	YES
25	8.8% CB-LS/0.3% LAO	4.1	60	YES	YES/borderline
26	7.7% CB-LS/0.3% LAO	3.6	60	NO	
27	6.6% CB-LS/0.2% LAO	3.1	60	NO	
28	9% CB	1.2	60	NO	
29	12% CAB/0.4% LAO	5.6	60	YES	YES
30	12% CAB	5.5	60	YES	YES
31	9.9% CAB	4.5	60	BL	Borderline
32	8.8% CAB	4.0	60	NO	—
33	7.7% CAB	3.5	60	NO	—
6	43.5% OB/2.2% LAO	11.9	60	YES	Borderline/clear[8]
34	35.4% OB/7.1% LAO	11.3	60	YES	YES/borderline
35	22.5% OB/4.5% LAO	9.0	60	YES	NO
36	25% OB/2.5% LAO	7.6	60	YES	NO
37	9% OB	1.2	60	NO	—
38	17.7% LAO	8.0	60	YES	YES
39	9% LAO	6.0	60	NO	—
46	9% LAO	6.0	RT	NO	—
40	21% LAO	1.2	60	NO	—
41	24% LB/0.8% LAO	6.5	60	YES	YES
42	12.5% LB/1.3% LAO	3.6	60	NO	—
43	37.7% OAO	11.1	60	NO	—
44	4.5% MAO/4.5% CAB	6.0	RT	NO	—

[4]Formulation numbers refer to the Formulations of the examples.
[5]Abbreviations of surfactants: CB-LS = cocobetaine, low salt version; LAO = lauryl amine oxide; CAB = cocamidopropyl betaine; OB = octyl betaine; LB = lauryl betaine; OAO = octyl amine oxide; MAO = myristyl amine oxide
[6]The weight ratio of total surfactants to triclosan.
[7]60° C. means 58–63° C.; RT means room temperature.
[8]Initially the Formulation was borderline upon sequential dilution but became a clear homogeneous solution upon standing at room temperature.

[0069] Table 1 shows that not only is a certain minimum ratio of total surfactant(s) to Triclosan necessary to obtain a clear homogeneous Formulation solution but also the antimicrobial agent—in this case Triclosan—must be introduced into the Formulation above its melting point, e.g., 57-58° C. for Triclosan. The clarity characteristics of the Formulations are shown in the last two columns of Table 1. It is also evident that some surfactants or surfactant combinations are more effective at giving clear homogeneous Formulations than other surfactants. It is well understood by those skilled in the art that all surfactants are not equally effective. For example, the cocobetaine—low salt version the weight ratio of the surfactant to Triclosan of about 4.1 is sufficient to give a clear homogeneous Formulation while for cocamidopropyl betaine, octyl amine oxide and lauryl oxide, higher levels of surfactant are required.

[0070] The last two columns of Table I sets forth data indicating how effectively Triclosan has been incorporated into the Formulation, how effectively the Formulation will transport Triclosan to take advantage of its antimicrobial activity, and potential antimicrobial efficacy of the Formulation. This analysis as detailed hereinbelow involves a visual inspection and therefore does not need any special or expensive equipment.

[0071] A. Analysis of the Initial Clarity:

[0072] After preparation, the Formulation is visually examined at room temperature to determine if it is a clear and homogeneous solution. A "Yes" classification is assigned if the Formulation is a clear and homogenous solution with no signs of solid particulates, oily material or any type of second phase. A "No" classification is assigned if the formulation is not clear, homogeneous, is a blue or white dispersion or emulsion or has a solid precipitate, or has an oily phase separated from the aqueous phase.

[0073] B. Analysis of the Clarity of the Sequentially Diluted Formulation

[0074] This involves visual examination of the formulation after sequential dilutions with water at ambient temperature. The analysis is carried out as follows:

[0075] To 1 gram of the Formulation being evaluated in a clear container, 4 grams of water are added to give a 1:5

dilution. The contents of the container are mixed manually for about one minute. The contents are visually inspected and assigned one of the following rankings: yes; yes/borderline; no/borderline; no. If a no or a no/borderline ranking is assigned, the analysis is complete and no further dilution steps are necessary. If a yes or yes/borderline ranking is assigned additional sequential dilutions by adding water to achieve the following dilutions:

[0076] 5 g water—1:10 dilution

[0077] 5 g water—1:15 dilution

[0078] 5 g water—1:20 dilution

[0079] 10 g water—1:30 dilution

[0080] 20 g water—1:50 dilution

[0081] The ranking assigned is given based on the following criteria:

[0082] Yes—the solution is clear and homogenous, no visual detection of solid or oil phase evident.

[0083] Yes/borderline—the solution has a very slight blue tint but has no signs of gross solid particles or oil phase.

[0084] No/borderline—the solution has a blue to slightly white appearance, the solution is no longer clear.

[0085] No—a white dispersion, emulsion or suspension is present, or solid has precipitated out, or a second liquid/oil phase is present.

[0086] The no or no/borderline ranking indicates that the Formulation is not sufficiently robust to deliver/transport the Triclosan and the Formulation will not be an effective antimicrobial. The Formulations that have been assigned a yes or yes/borderline ranking have a level of surfactant present which is sufficient to both keep the Triclosan in aqueous solution (prevent phase separation) and to deliver/transport the Triclosan effectively and give an efficacious antimicrobial as shown in Table 2. In those cases where sufficient surfactant is present, there is no need to incorporate solvents to dissolve the Triclosan into the Formulation. In addition there is no need to incorporate hydrotropes to aid in the delivery/transport of the Triclosan in the antimicrobial Formulation. It is highly desirable to eliminate solvents from Triclosan antimicrobial Formulation because to their toxicity, VOC (volatile organic compound) pollution and fire hazard issues, and irritation characteristics. Many of these solvents are harsh to human skin and eyes and are detriments to antimicrobial Formulations. In addition the present of solvents poses hazards and limits the useful utility in large scale, household, commercial, institutional and industrial settings. In addition it is highly desirable to eliminate hydrotropes from antimicrobial Formulations. Hydrotropes are very strong acids or salts that are irritants, corrosive and harsh to human skin and eyes. The present invention eliminates the need to use solvents and/or hydrotropes in antimicrobial Formulation by incorporating the Triclosan into the Formulation above the melting point of Triclosan or by using a second liquid antimicrobial agent to dissolve/liquefy the Triclosan and by using a sufficient amount of surfactant to both incorporate the Triclosan into the Formulation and to keep the Triclosan in the Formulation upon dilution. These features are indicative of effective delivery/transport of the Triclosan to give an efficacious antimicrobial. The surfactants used in the present invention are preferably very mild and can mitigate irritation to human skin and eyes.

TABLE 2

Antimicrobial Efficacy for Clear and Homogeneous Formulations

Formulation	Zone of Inhibition (mm)		Plate Cidality	
No.	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Formulation 5	52	50	YES	YES
Formulation 1	51	42	YES	YES
Formulation 2	53	42	YES	YES
Formulation 6	45	37	YES	YES

S. aureus = *Staphylococcus aureus*,
E. coli = *Escherichia coli*

[0087] Table 2 shows that antimicrobial Formulations which are initially clear and homogeneous and remain clear and homogeneous upon sequential dilution have a high degree of antimicrobial efficacy. While the prior art has employed high levels of hydrotropes or combinations of solvent and hydrotropes, we have achieved exceptional antimicrobial efficacy with a combination of the right level of surfactant and the right processing temperature in a easy straight forward process. Elimination of solvents and hydrotropes is significant since these materials can be flammable, toxic, corrosive or irritants to the skin and eyes thereby limiting broad industrial, commercial, institutional and household use.

TABLE 3

Inadequate Antimicrobial Efficacy for Formulations Which Are Not Clear and Homogeneous

Formulation	Zone of Inhibition (mm)		Plate Cidality	
No.	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
34	45	37	NO	YES
35	42	33	NO	YES
39	49	35	NO	YES
46	42	36	NO	YES
43	38	30	NO	YES
44	49	43	NO	NO

S. aureus = *Staphylococcus aureus*,
E. coli = *Escherichia coli*

[0088] Table 3 shows that antimicrobial Formulations which are not initially clear and homogeneous or do not remain clear and homogeneous upon sequential dilution have inadequate antimicrobial efficacy. These Formulations have smaller zones of inhibition and fail to kill *Staphylococcus aureus* or fail to kill both *Staphylococcus aureus* and *Escherichia coli*. These antimicrobial Formulations lack broad efficacy. Those skilled in the art can determine the minimum required surfactant level by using the techniques described above to determine both the initial Formulation clarity and homogeneity and the clarity and homogeneity of sequentially diluted material on a series of Formulations where the surfactant or surfactant combination has been incrementally increased over a series of Formulations. The minimum level of surfactant necessary is determined at the point where both the Formulation and sequentially diluted Formulations are both clear and homogeneous.

[0089] In addition, the antimicrobial Formulations of the present invention are effective at high dilution. Table 4

shows the Minimum Inhibitory Concentration (MIC) and the Minimum Bacteriacidal Concentration (MBC) values determined for two of the Formulations of the present invention against four microbes and compared to a commercial antibacterial hand soap containing Triclosan. The MIC values were determined using a standard broth method with sequential two fold dilutions. The MIC's were determined by visual inspection at the point of no turbidity.

TABLE 4

Minimum Inhibitory Concentrations

Antimicrobial	Organism	MIC/MBC
Dial ® Antibacterial Soap	*Staphylococcus epidermidis*	<1:10/static
	Corynebacterium jeikeium	1:10/static
	Micrococcus luteus	<1:10/static
	Propionibacterium acnes	<1:10/static
Formulation 10	*Staphylococcus epidermidis*	>1:1280/>1:1280
	Corynebacterium jeikeium	>1:1280/>1:1280
	Micrococcus luteus	1:640/1:320
	Propionibacterium acnes	1:640/1:320
Formulation 14	*Staphylococcus epidermidis*	>1:1280/>1:1280
	Corynebacterium jeikeium	>1:1280/>1:1280
	Micrococcus luteus	>1:1280/>1:1280
	Propionibacterium acnes	>1:1280/>1:1280

[0090] The first entry above in Table 4 is a commercial antibacterial soap containing Triclosan. It was used as purchased with the first dilution being 1:10. The results preceded by <means the dilution was too great and the MIC was passed. The results preceded by> means the test was stopped but further dilution of the Formulation is still possible. As you can see the efficacy of the two Formulations of the present invention is far superior to the Triclosan commercial comparison. In addition our two Formulations were also cidal to the microorganisms at high dilution (MBC).

[0091] The antimicrobial Formulations of the present invention can be used in the concentrated form as prepared or diluted until the minimum concentration which still maintains efficacy is reached. Table 4 shows that dilutions greater than 1:1000 are achievable in the present invention.

[0092] Table 5 shows the superior antimicrobial efficacy of Formulations of the present invention compared to a commercial kitchen cleaner, commercial alcohol gel, commercial handsoap with Triclosan, chlorhexidine and betadine solutions.

TABLE 5

Antimicrobial Zone of Inhibition

	Zones of Inhibition (mm)	
Antimicrobial Agent	*Staphylococcus aureus*	*Pseudomonas aeruginsoa*
Lysol ® kitchen Cleaner	24	CI
Alcohol Gel	Not tested	CI
Commercial Handsoap with Triclosan	Not tested	CI
4% chlorohexidine	Not tested	16
10% Betadine	Not tested	14
Formulation 1	51	20
Formulation 2	53	14
Formulation 3	41	16
Formulation 4	50	14
Formulation 5	52	18
Formulation 6	45	14
Formulation 7	57	43
Formulation 8	112	72
Formulation 9	40	16
Formulation 10	61	29
Formulation 11	61	29
Formulation 12	24	Not tested
Formulation 13	61	31
Formulation 14	110	70
Formulation 15	100	48
Formulation 16	102	52
Formulation 17	60	41
Formulation 18	110	70
Formulation 19	98	55

CI = contact inhibition only, no Zone of Inhibition detected.

[0093] The results in Table 5 show the superior efficacy of the antimicrobial Formulations of the present invention

[0094] The following working examples describe presently-preferred embodiments of the present invention.

[0095] In the examples, the antimicrobial testing was carried out as follows:

[0096] Determination of Zone of Inhibition

[0097] Disks impregnated with known concentrations of antimicrobials are placed onto the surface of Mueller-Hinton Agar 150 mm plates that have been freshly seeded with a known quantity of bacteria (varying from 10/5 to 10/8 cfus/ml). Numerous different bacteria can be challenged in this manner in order to determine the spectrum of activity of the agent. The standard inoculum is a 0.5 Mc Farland standard turbidity, which is approximately 1.5×10/8 cfu/ml. The surface of the agar is swabbed in three directions to ensure an even and complete distribution of inoculum over the entire plate. Within 15 minutes of inoculation the antimicrobial agent disk disks are applied and the plates are inverted for incubation to avoid accumulation of moisture on the agar surface that might interfere with the interpretation of the test results. For most organisms, incubation is at 35 C in air but increased CO_2 is used with certain fastidious bacteria. The dynamics and timing of antimicrobial agent diffusion to establish a concentration gradient coupled with the growth of organisms over an 18- to 24-hour duration is critical for reliable results. Therefore incubation of disks beyond that allotted time should be avoided.

[0098] Using a dark background and a reflected light the plate is situated so that a ruler or caliper may be used to measure inhibition zone diameters for each antimicrobial agent.

[0099] Zones are recorded in millimeters (mm) of diameter. Known controls (including media controls) are employed with each run to assure accuracy of techniques employed. This Zone of Inhibition test was conducted using the formulations in the examples diluted 1 to 3 with water.

[0100] Plate Cidality

[0101] In order to determine whether the antimicrobial agent being tested had Plate Cidality vs no Plate Cidality, a loop full of inoculum was taken within the Zone of Inhibition approximately 2 mm from the disk edge after the plates have been incubated for 18-24 hours. The loop was inoculated to a fresh Trypticase Soy Agar plate and then incubated at 35 C for 18-24 hours before being read. The results were recorded as "No growth", indicating Plate Cidality or "Growth" indicating only no Plate Cidality. Alternatively the number of colonies was recorded in cases where there was incomplete Plate Cidality.

[0102] Testing for Yeast and Mold Fungi

[0103] Disks impregnated with known concentrations of antimicrobials are placed onto the surface of Sabouraud Dextrose Agar 150 mm plates that have been freshly seeded with a known quantity of fungi. Numerous different yeast and mold fungi can be challenged in this manner in order to determine the spectrum of activity of the agent. The standard inoculum is a 0.5 Mc Farland standard turbidity, which is approximately 1.5×10/8 cfu/ml. This concentration is used for yeast fungi. For mold fungi spores are harvested in PBS and using a hemocytometer an approximate count is made guaranteeing an inocula of at least 1.5×10/4 to 10/5 cfu/ml. The surface of the agar is swabbed in three directions to ensure an even and complete distribution of inoculum over the entire plate. Within 15 minutes of inoculation, the antimicrobial agent disk disks are applied and the plates are inverted for incubation to avoid accumulation of moisture on the agar surface that might interfere with the interpretation of the test results. For most organisms, incubation is at 30-35 C in ambient air dependent upon the fungal species. The dynamics and timing of antimicrobial agent diffusion to establish a concentration gradient coupled with the growth of organisms over a 24-48 hours duration for yeast fungi and up to 10 days for mold fungi is critical for reliable results.

[0104] Using a dark background and a reflected light the plate is situated so that a ruler or caliper may be used to measure inhibition zone diameters for each antimicrobial agent.

[0105] Zones are recorded in millimeters (mm) of diameter. Known controls (including media controls) are employed with each run to assure accuracy of techniques employed.

[0106] Plate Cidality for Fungi

[0107] In order to determine whether the antimicrobial agent being tested had Plate Cidality vs static, a loopful of inoculum was taken within the Zone of Inhibition approximately 2-4 mm from the disk edge after the plates have been incubated for the designated time. The loop is inoculated to a Sabauroud Dextrose Agar plate and then incubated at 30-5 C dependent upon the fungus for the appropriate time dependent upon whether it is a mold or yeast fungus before being read. The results are recorded as "No growth", indicating Plate Cidality or "Growth" indicating only no Plate Cidality. Alternatively the number of colonies are to be recorded in cases where there was incomplete Plate Cidality.

[0108] Shell Vial Culture for Viral Analysis

[0109] MRC-5 human embryonic diploid lung fibroblast cells are inoculated with a previously germicide treated ATCC culture of Herpes Simplex virus maintained in Eagle's minimum essential medium. The treatment is with a germicide to be tested at a 1:3 dilution held at room temperature for 1 hour prior to cell inoculation. Two shell vials are inoculated in this manner and then incubated at 35 C, one for 24 hours and the other for 48 hours. After incubation they are observed for cytopathic effect (CPE) using standard immunofluorescent staining. CPE negative is considered to be an effective kill of HSV. CPE positive is considered to be a failure. Positive and negative controls are run with all experiments.

EXAMPLE 1

Formulation 1

[0110] The Formulation described in the present example contained the following components:

Component	Mass (grams)
Water	53.27
Coco betaine - low salt	42.8
lauryl amine oxide	1.33
citric acid	0.4
Triclosan	2.2

[0111] The total mass of the Formulation is 100 grams.

[0112] A 250 ml beaker equipped with a magnetic stir bar is charged with 53.27 grams water, 42.8 grams coco betaine—low salt, 1.33 g lauryl amine oxide and 0.4 g citric acid. The contents of the beaker are stirred and heated to 60° C. At 61° C. 2.2 grams of Triclosan are added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at 61-63° C. for 20 minutes. The Formulation is cooled to room temperature and has a pH of about 4.6. One gram of the clear homogenous solution is added to 4 grams of water and a clear homogenous solution forms for a 1:5 dilution. An additional 5 grams of water is added to this clear solution and a clear homogenous solution forms for a 1:10 dilution. Sequential dilutions are continued up to 1:50 dilution with each dilution still giving a clear homogenous solution.

[0113] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 51 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 42 millimeter Zone of Inhibition for *Escherichia coli* and a 29 millimeter Zone of Inhibition for *Enterococcus* The Formulation also displayed bacteriocidal properties for both *S. aureus* and *E. coli* after a 10 minute exposure to the Formulations at all dilutions. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 2

Formulation 2

[0114] A Formulation was prepared according to the method of Example 1, but contained the following components:

Component	Mass (grams)
Water	26.38
Coco betaine - low salt	21.40
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
Zonyl FS510	0.5

[0115] The total mass of the Formulation is 50.25 grams. Zonyl FS510 was added after the Triclosan. The initial Formulation was clear and the dilutions were also clear. 4

[0116] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 53 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 42 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 3

Formulation 3

[0117] A Formulation was prepared according to the method of Example 1, but contained the following components:

Component	Mass (grams)
Water	22.49
Coco betaine - low salt	21.40
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
ammonium bifluoride	1.2
Phosphoric acid	2.94

[0118] The total mass of the Formulation is 50 grams. The ammonium bifluoride and phosphoric acid were added to the Formulation after the Triclosan at approximately 60° C. The initial Formulation was clear and the dilutions were also clear.

[0119] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 41 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 30 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 4

Formulation 4

[0120] A Formulation was prepared according to the method of Example 1, but contained the following components:

Component	Mass (grams)
Water	53.27
Coco betaine - low salt	42.8
lauryl amine oxide	1.33
citric acid	0.4
Triclosan	2.2

[0121] The total mass of the Formulation is 100 grams. The initial Formulation was clear and the dilutions were also clear.

[0122] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 50 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 39 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 5

Formulation 5

[0123] A high solids Formulation was prepared according to the method of Example 1, but contained the following components:

Component	Mass (grams)
Coco betaine - low salt	42.8
lauryl amine oxide	1.33
citric acid	0.4
Triclosan	2.2

[0124] The total mass of the Formulation is 46.73 grams. The initial Formulation was clear and the dilutions were also clear.

[0125] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 52 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 50 millimeter Zone of Inhibition for *Escherichia coli* and a 31 millimeter Zone of Inhibition for *Enterococcus* The Formulation also displayed bacteriocidal properties for both *S. aureus* and *E. coli* after a 10 minute exposure to the Formulations at all dilutions. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 6

Formulation 6

[0126] A high solids Formulation was prepared according to the method of Example 1, but contained the following components:

Component	Mass (grams)
Octylbetaine	25.0
lauryl amine oxide	2.09

-continued

Component	Mass (grams)
citric acid	0.15
sodium citrate	0.41
Triclosan	1.1

[0127] The total mass of the Formulation is 28.75 grams. The initial Formulation was clear and the dilutions were also clear.

[0128] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 45 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 37 millimeter Zone of Inhibition for *Escherichia coli* and a 29 millimeter Zone of Inhibition for *Enterococcus*. The Formulation also displayed bacteriocidal properties for both *S. aureus* and *E. coli*. after a 10 minute exposure to the Formulations at all dilutions. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 7

Formulation 7

[0129] A Formulation was prepared as described below and contained the following components:

Component	Mass (grams)
Water	11.63
Cocobetaine - low salt	21.40
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
Hydrogen peroxide (30%)	15

[0130] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0131] A 250 ml beaker equipped with a magnetic stir bar is charged with 11.63 grams water, 21.4 grams coco betaine—low salt, 0.66 grams lauryl amine oxide, and 0.2 grams citric acid. The contents of the beaker are stirred and heated to 60° C. At 61° C. 2.2 grams of Triclosan are added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at 59-63° C. for 20 minutes. The Formulation is cooled to room temperature (22° C.) and 15 grams of hydrogen peroxide are added. The solution is stirred for 10 minutes. The clear homogenous low viscosity solution has a pH of about 4.8. One gram of the clear homogenous solution is added to 4 grams of water and a clear homogenous solution forms for a 1:5 dilution. An additional 5 grams of water is added to this clear solution and a clear homogenous solution forms for a 1:10 dilution. Sequential dilutions are continued up to 1:50 dilution with each dilution still giving a clear homogenous solution. Testing of the Formulation confirms this and shows it has superior antimicrobial efficacy.

[0132] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 57 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 43 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 8

Formulation 8

[0133] A Formulation was prepared as described above in Example 7 and contained the following components:

Component	Mass (grams)
Water	20.2
Cocobetaine - low salt	21.40
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
t-butylhydroperoxide (70%)	6.43

[0134] The total mass of the Formulation is 50 grams. The initial Formulation was mostly clear and the dilutions were also clear.

[0135] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 112 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 106 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 9

Formulation 9

[0136] A Formulation was prepared as described above as in Example 7 and contained the following components:

Component	Mass (grams)
Water	44.67
Cocobetaine - low salt	42.8
lauryl amine oxide	1.33
citric acid	9.0
Triclosan	2.2

[0137] The total mass of the Formulation is 100 grams. The initial Formulation was clear and the dilutions were also clear.

[0138] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 40.5 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 33 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 10

Formulation 10

[0139] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	5.76
Coco betaine - low salt	21.4
lauryl amine oxide	1.0
mysrtyl amine oxide	1.0
cocamidopropyl amine oxide	1.34
citric acid	4.5
Hydrogen peroxide (30%)	15

[0140] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0141] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 61 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 45 millimeter Zone of Inhibition for *Escherichia coli* and a 29 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 11

Formulation 11

[0142] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	7.0
Coco betaine - low salt	21.40
lauryl amine oxide	1.0
citric acid	4.5
Triclosan	1.1
Hydrogen peroxide (30%)	15

[0143] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0144] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 60.5 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 44 millimeter Zone of Inhibition for *Escherichia coli* and a 29 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 12

Formulation 12

[0145] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	20.76
Coco betaine - low salt	21.4
lauryl amine oxide	1.0
myristyl amine oxide	1.0
cocamidopropyl amine oxide	1.34
citric acid	4.5

[0146] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0147] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 24 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 19 millimeter Zone of Inhibition for *Escherichia coli.*

EXAMPLE 13

Formulation 13

[0148] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	4.66
Coco betaine - low salt	21.4
lauryl amine oxide	1.0
myristyl amine oxide	1.0
cocamidopropyl amine oxide	1.34
citric acid	4.5
Triclosan	1.1
Hydrogen peroxide (30%)	15

[0149] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0150] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 61 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 44 millimeter Zone of Inhibition for *Escherichia coli* and a 31 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 14

Formulation 14

[0151] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	5.92
Cocobetaine - low salt	35.68
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
t-butylhydroperoxide (70%)	6.43

[0152] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0153] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 110 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 110 millimeter Zone of Inhibition for *Escherichia coli* and a 70 millimeter Zone of Inhibition for *Pseudomonas aeruginosa* and a 36 millimeter Zone of Inhibition for *Candida albicans* and a 49 millimeter Zone of Inhibition for *Aspergillus niger* and a 28 millimeter Zone of Inhibition for *Mycobacterium fortuitum* and a 74 millimeter Zone of Inhibition for methicillin resistant *Staphylococcus aureus* and a Zone of Inhibition of 35 millimeters for vancomycin-resistant Enterococci and a Zone of Inhibition of 100 millimeters for mulit-drug resistant *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for all these nine microorganisms. Herpes Simplex cultures were tested against this formulation after exposing it to this formulation for 15 minutes before being cultured and then subjected to the shell vial culturing methodology. There was no growth observed after 24 and 48 hours with this formulation while good growth was observed in the controls after both 24 and 48 hours.

EXAMPLE 15

Formulation 15

[0154] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	16.71
Cocobetaine - low salt	25.0
lauryl amine oxide	0.69
citric acid	0.22
Triclosan	1.1
t-butylhydroperoxide (70%)	6.42

[0155] The total mass of the Formulation is 50.14 grams. The initial Formulation was clear and the dilutions were also clear.

[0156] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 100 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 110 millimeter Zone of Inhibition for *Escherichia coli* and a 48 millimeter Zone of Inhibition for *Pseudomonas aeruginosa.*

EXAMPLE 16

Formulation 16

[0157] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	13.77
Cocobetaine - low salt	21.4
lauryl amine oxide	7.1
citric acid	0.2
Triclosan	1.1
t-butylhydroperoxide (70%)	6.43

[0158] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0159] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 102 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 100 millimeter Zone of Inhibition for *Escherichia coli* and a 52 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 17

Formulation 17

[0160] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	22.35
Cocobetaine - low salt	23.54
lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
t-butylhydroperoxide	2.14

[0161] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0162] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 60 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 56 millimeter Zone of Inhibition for *Escherichia coli* and a 41 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 18

Formulation 18

[0163] A Formulation was prepared as described above as in Example 7. The Formulation contained the following components:

Component	Mass (grams)
Water	13.06
Cocobetaine - low salt	28.54
Lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
t-butylhydroperoxide (70%)	6.43

[0164] The total mass of the Formulation is 50 grams. The initial Formulation was clear and the dilutions were also clear.

[0165] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 110 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 110 millimeter Zone of Inhibition for *Escherichia coli* and a 70 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 19

Formulation 19

[0166]

Component	Mass (grams)
Water	21.30
Lauryl amine oxide	0.67
cocobetaine	21.40
citric acid	0.2
t-butylhydroperoxide	6.43

[0167] The total mass of the Formulation is 50 grams.

[0168] Formulation 19 was prepared by sequentially mixing the components in the order listed at room temperature. Total mixing time at room temperature was about 25 minutes.

[0169] Testing of the Formulation shows that it has superior antimicrobial efficacy. In the zones of inhibition test, this Formulation caused a 98 millimeter Zone of Inhibition for *Staphylococcus aureus* and an 85 millimeter Zone of Inhibition for *Escherichia coli* and a 55 millimeter Zone of Inhibition for *Pseudomonas aeruginosa*. In the Plate Cidality test there was no growth observed for these three microorganisms.

EXAMPLE 20

Formulation 20

[0170] This example demonstrates that by selecting a liquid antimicrobial agent that can dissolve or liquefy the second solid antimicrobial agent, it is not necessary employ heat in the Formulation process to give a clear, homogeneous Formulation which is also clear and homogeneous upon sequential dilution. These clear and homogeneous Formulations have a high degree of antimicrobial efficacy. The Formulation was prepared as described below and contained the following components:

Component	Mass (grams
t-butyl hydroperoxide (70%)	12.86
Triclosan	2.20
Cocobetaine - low salt	71.36
Citric acid	0.40
Aqueous 2000 ppm iron sulfate solution	2.00
Water	11.18

[0171] The total mass of the Formulation is 100 grams. The initial Formulation was clear and homogeneous and the dilutions were also clear and homogeneous.

[0172] The following procedure was used to prepare this Formulation:

[0173] A 250 ml beaker equipped with a magnetic stir bar was charged with 12.86 grams of t-butyl hydroperoxide (70%) and 2.20 grams of Triclosan. The solid—liquid dispersion was stirred at room temperature. After about 10 to 15 minutes of stirring an all liquid mixture forms. To the two phase oil and water mixture is added 71.36 grams of cocobetaine—low salt version—and stirred at room temperature. After about 2-5 minutes a clear and homogeneous solution forms. To the solution is added 0.40 grams of citric acid and stirring is continued at room temperature for 30 minutes. To the clear and homogeneous solution are added 2.00 grams of an aqueous solution containing 2000 ppm of iron sulfate followed by 11.18 grams of water. The solution is stirred an additional 10 minutes. The clear homogeneous solution has a low viscosity and a pH of 6.5. One gram of the clear homogeneous solution is added to 4 grams of water and a clear homogeneous solution forms for a 1:5 dilution. An additional 5 grams of water are added to this clear solution and a clear homogeneous solution forms for a 1:10 dilution. Sequential dilutions are continued up to 1:50 dilution with each dilution giving a clear homogeneous solution.

EXAMPLE 21

[0174] Two Herpes Simplex ATCC (#vr-733) cultures were tested against Formulation 14 for (eliminate: 12 for) 15 minutes before being cultured) and then subjected to shell vial culturing as outlined in methodology below. The result was that each was CPE negative in that no growth in either culture after exposure to Formulation 14 after 24 and 48

hours, was observed while good growth was observed in controls after both 24 and 48 hours."

[0175] Examples 22-43 were prepared using the following procedure:

[0176] A 250 ml beaker equipped with a magnetic stir bar is charged with water (if used in the formulation), surfactant, citric acid, sodium citrate (if used in the formulation) and a fluorocarbon wetting agent (if used in the formulation. The contents of the beaker are stirred and heated to about 60-63° C. At about 60° C. Triclosan is added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at about 60-63° C. for 20 minutes. The Formulation is cooled to room temperature and has a pH in the range of about 4.0-7.0.

EXAMPLE 22

[0177] Example 22 contained the following components:

Component	Mass (grams)
Water	0.00
Coco betaine - low salt	22.3
lauryl amine oxide	2.1
citric acid	0.15
Sodium citrate	0.41
Triclosan	1.0

[0178] The total mass of the Formulation is 25.96 grams and a pH of 5.5.

EXAMPLE 23

[0179] Example 23 contained the following components:

Component	Mass (grams)
Water	54.6
Coco betaine - low salt	42.8
citric acid	0.40
Triclosan	2.2

[0180] The total mass of the Formulation is 100.0 grams and a pH of 4.5.

EXAMPLE 24

[0181] Example 24 contained the following components:

Component	Mass (grams)
Water	0.00
Coco betaine - low salt	28.6
Lauryl amine oxide	1.33
citric acid	0.40
Triclosan	1.6

[0182] The total mass of the Formulation is 31.93 grams and a pH of 4.7.

EXAMPLE 25

[0183] Example 25 contained the following components:

Component	Mass (grams)
Water	64.99
Coco betaine - low salt	31.43
Lauryl amine oxide	0.98
citric acid	0.40
Triclosan	2.2

[0184] The total mass of the Formulation is 100.0 grams and a pH of 4.3.

EXAMPLE 26

[0185] Example 26 contained the following components:

Component	Mass (grams)
Water	69.04
Coco betaine - low salt	27.5
Lauryl amine oxide	0.86
citric acid	0.40
Triclosan	2.2

[0186] The total mass of the Formulation is 100.0 grams and a pH of 4.2.

EXAMPLE 27

[0187] Example 27 contained the following components:

Component	Mass (grams)
Water	36.69
Coco betaine - low salt	11.77
Lauryl amine oxide	0.36
citric acid	0.21
Triclosan	1.1

[0188] The total mass of the Formulation is 50.13 grams and a pH of 4.2.

EXAMPLE 28

[0189] Example 28 contained the following components:

Component	Mass (grams)
Water	65.9
Coco betaine - low salt	26.6
Triclosan	7.5

[0190] The total mass of the Formulation is 100.0 grams.

EXAMPLE 29

[0191] Example 29 contained the following components:

Component	Mass (grams)
Water	56.07
Cocamidopropyl betaine	40.0
Lauryl amine oxide	1.33
citric acid	0.40
Triclosan	2.2

[0192] The total mass of the Formulation is 100.0 grams and a pH of 4.4.

EXAMPLE 30

[0193] Example 30 contained the following components:

Component	Mass (grams)
Water	57.4
Cocamidopropyl betaine	40.0
citric acid	0.40
Triclosan	2.2

[0194] The total mass of the Formulation is 100.0 grams and a pH of 4.0.

EXAMPLE 31

[0195] Example 31 contained the following components:

Component	Mass (grams)
Water	64.4
Cocamidopropyl betaine	33.0
citric acid	0.40
Triclosan	2.2

[0196] The total mass of the Formulation is 100.0 grams and a pH of 4.0.

EXAMPLE 32

[0197] Example 32 contained the following components:

Component	Mass (grams)
Water	68.07
Cocamidopropyl betaine	29.33
citric acid	0.40
Triclosan	2.2

[0198] The total mass of the Formulation is 100.0 grams and a pH of 4.0.

EXAMPLE 33

[0199] Example 33 contained the following components:

Component	Mass (grams)
Water	71.73
Cocamidopropyl betaine	25.67
citric acid	0.40
Triclosan	2.2

[0200] The total mass of the Formulation is 100.0 grams and a pH of 4.0.

EXAMPLE 34

[0201] Example 34 contained the following components:

Component	Mass (grams)
Water	0.00
Octyl betaine	22.5
Lauryl amine oxide	7.5
citric acid	0.15
Sodium citrate	0.41
Triclosan	1.2

[0202] The total mass of the Formulation is 31.76 grams and a pH of 6.3. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

EXAMPLE 35

[0203] Example 35 contained the following components:

Component	Mass (grams)
Water	17.12
Octyl betaine	22.5
Lauryl amine oxide	7.5
citric acid	0.3
Sodium citrate	0.83
Triclosan	1.5

[0204] The total mass of the Formulation is 49.75 grams and a pH of 6.0. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

EXAMPLE 36

[0205] Example 36 contained the following components:

Component	Mass (grams)
Water	18.47
Octyl betaine	25.0
Lauryl amine oxide	4.17
citric acid	0.15
Sodium citrate	0.41
Triclosan	1.8

[0206] The total mass of the Formulation is 50.0 grams and a pH of 6.5.

EXAMPLE 37

[0207] Example 37 contained the following components:

Component	Mass (grams)
Water	35.87
Octyl betaine	9.0
citric acid	0.3
Sodium citrate	0.83
Zonyl ® FSN 100	0.25
Triclosan	3.75

[0208] The total mass of the Formulation is 50.0 grams and a pH of 5.0. Zonyl® FSN 100 is a fluorocarbon wetting agent available from DuPont.

EXAMPLE 38

[0209] Example 38 contained the following components:

Component	Mass (grams)
Water	19.54
Lauryl amine oxide	29.16
citric acid	0.2
Triclosan	1.1

[0210] The total mass of the Formulation is 50.0 grams and a pH of 6.0.

EXAMPLE 39

[0211] Example 39 contained the following components:

Component	Mass (grams)
Water	64.25
Lauryl amine oxide	30.0
citric acid	0.6
Sodium citrate	1.65
Zonyl ® FSN 100	0.5
Triclosan	1.5
Ammonium bifluoride	1.5

[0212] The total mass of the Formulation is 100.0 grams and a pH of 4.7. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

EXAMPLE 40

[0213] Example 40 contained the following components:

Component	Mass (grams)
Water	2.5
Lauryl amine oxide	30.0
citric acid	0.6
Sodium citrate	1.65
Zonyl ® FSN 100	0.5
Triclosan	7.5

[0214] The total mass of the Formulation is 42.75 grams and a pH of 6.1.

EXAMPLE 41

[0215] Example 41 contained the following components:

Component	Mass (grams)
Water	3.31
lauryl betaine	42.9
Lauryl amine oxide	1.33
citric acid	0.15
Sodium citrate	0.41
Triclosan	1.9

[0216] The total mass of the Formulation is 50.0 grams and a pH of 5.5.

EXAMPLE 42

[0217] Example 42 contained the following components:

Component	Mass (grams)
Water	23.14
lauryl betaine	22.3
Lauryl amine oxide	2.1
citric acid	0.15
Sodium citrate	0.41
Triclosan	1.9

[0218] The total mass of the Formulation is 50.0 grams and a pH of 6.0.

EXAMPLE 43

[0219] Example 43 contained the following components:

Component	Mass (grams)
Water	0.00
octyl amine oxide	25.00
citric acid	0.15
Sodium citrate	0.41
Triclosan	0.9

[0220] The total mass of the Formulation is 26.46 grams and a pH of 7.0. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

[0221] Examples 44-46 were prepared using the following procedure:

[0222] A 250 ml beaker equipped with a magnetic stir bar is charged with water, surfactant, citric acid, and sodium citrate (if used in the formulation). At room temperature the contents of the beaker are stirred while the components are sequentially added at about one minute apart. Upon completion of the addition of the components the formulation is stirred at room temperature for about 30 minutes.

EXAMPLE 44

[0223] Example 44 contained the following components:

Component	Mass (grams)
Water	66.05
cocamidopropyl betaine	15.0
myristamineamine oxide	15.0
citric acid	0.8
Sodium citrate	1.65
Triclosan	1.5

[0224] The total mass of the Formulation is 100.0 grams and a pH of 5.5. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

EXAMPLE 45

[0225] Example 45 contained the following components:

Component	Mass (grams)
Water	53.27
cocobetaine - low salt version	42.8
Lauryl amine oxide	1.33
citric acid	0.4
Triclosan	2.2

[0226] The total mass of the Formulation is 100.0 grams and a pH of 4.7. The antimicrobial effectiveness for this composition is shown in Table 3 hereinabove.

EXAMPLE 46

[0227] Example 46 contained the following components:

Component	Mass (grams)
Water	56.75
Lauryl amine oxide	30.0
citric acid	3.0
Sodium citrate	8.25
Zonyl ® FSN 100	0.5
Triclosan	1.5

[0228] The total mass of the Formulation is 100.0 grams and a pH of 6.0.

EXAMPLE 47

[0229] Example 47 contained the following components:

Component	Mass (grams)
Water	11.63
cocobetaine - low salt version	21.4
Lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
Peracetic acid - 30%	15.0

[0230] The total mass of the Formulation is 50.0 grams and a pH of 3.0.

[0231] Example 47 was prepared using the following procedure:

[0232] A 250 ml beaker equipped with a magnetic stir bar is charged with 11.63 grams of water, 21.4 grams of cocobetaine—low salt version, 0.67 grams of lauryl amine oxide, 0.2 grams of citric acid, The contents of the beaker are stirred and heated to about 60-63° C. At about 60° C. 1.1 grams of Triclosan are added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at about 60-63° C. for 20 minutes. The Formulation is cooled to room temperature and 15.0 grams of 30% peracetic acid are added with stirring. The solution is stirred an additional 10 minutes at room temperature. The formulation has a pH of about 3.0. The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution.

EXAMPLE 48

[0233] Example 48 demonstrates an ultra high % active ingredient and % solids antimicrobial formulation. Example 48 contained the following components:

Component	Mass (grams)
Lauramidopropyl betaine - powder	12.48
t-butyl hydroperoxide	3.10

[0234] Formulation 48 was prepared by the following procedure:

[0235] To a beaker containing 12.48 grams of powder lauramidopropyl betaine (98%) was added 3.10 grams of t-butyl hydroperoxide (70%). The contents of the beaker were manually stirred with a spatula at room temperature for 5 minutes to a uniform and homogeneous paste. The formulation has a % active ingredient of 92.4% and a % solid of 78.5%. By reducing the amount of t-butyl hydroperoxide to 1.00 grams a 97.8% active ingredient and 90.7% solids formulation can be prepared.

[0236] The 98% powder lauramidopropyl betaine is available from the Mcintyre Group Ltd. as Macham® 1200.

[0237] The other surfactants used in the above examples are available from the following commercial sources:

[0238] Cocobetaine, low salt version, from Mcintyre Group Ltd as Macham® CB35ULSHP, 28%

[0239] Lauryl amine oxide from Stepan Company as Ammonyx® LO, 30%

[0240] Cocamidopropyl betaine from Stepan Company as Amphosol® CA. 30%

[0241] Octyl betaine from Mcintyre Group Ltd as Macham® OCTLS, 50%

[0242] Lauryl betaine from Mcintyre Group Ltd as Macham® LB35, 28%

[0243] Octyl amine oxide from Lonza Inc. as FMB AO-8, 40%

[0244] Myristyl amine oxide from Stepan Company as Ammonyx® MO, 30%

[0245] Fluorocarbon wetting agents Zonyl® FSN 100 and Zonyl® FS 510 from Dupont.

EXAMPLE 49

[0246] This example illustrates an ultra high solids Antimicrobial Formulation

[0247] The formulation contained the following components:

Component	Mass (grams)
Cocobetaine - solid 98%	5.03
Triclosan	1.12

[0248] Total mass of the formulation is 6.15 grams.

[0249] An aluminum weighing pan of approximately 2 inches in diameter containing 5.03 grams of solid, brittle waxy cocobetaine was place on a hot plate set at 100° C. On top of the cocobetaine solid was placed 1.12 grams of Triclosan. As the aluminum pan was heated the Triclosan melted and softened the cocobetaine. Once the Triclosan was completely melted the mixture was stirred while hot for 5 minutes with a spatula. A homogeneous off white paste formed which remained as a paste when cooled to room temperature. The off white paste had a solids of about 98.4% with about 18.2% Triclosan.

[0250] The solid cocobetaine was obtained from the commercially available aqueous solution (Macham CB35ULSHP) by air drying 14.37 grams of the aqueous solution in an aluminum weighing pan for 5 days at room temperature in a fume hood. The aluminum weighing pan containing the air dried cocobetaine was placed on a hot plate set at 100° C. for 5 minutes during which time a constant mass was achieved. The solid brittle/waxy cocobetaine was used in the above formulation.

EXAMPLE 50

Formulation 50

[0251] This example illustrates an antimicrobial Formulation with a High Level of t-butyl Hydroperoxide with Iron Sulfate Present.

[0252] The first component of the formulation was prepared as described above as in Example 7.

[0253] The formulation contained the following two components:

Component	Mass (grams)
Water	3.27
Cocobetaine - low salt version	57.16
Lauryl amine oxide	1.34
Citric acid	0.40
Triclosan	2.20
Mixture of cocobetaine and t-butyl hydroperoxide	14.20 cocobetaine 21.43 t-butyl hydroperoxide

[0254] Total mass of Component 1 is 100 grams.

[0255] Component 2 consists of 20 grams of aqueous iron sulfate with an iron sulfate level of 20 ppm.

[0256] The final step in preparing this formulation consisted of taking a 10 gram portion of Component 1 and adding it to Component 2, the 20 grams of iron sulfate solution, and stirring at room temperature for 10 minutes.

[0257] Testing of the formulation this formulation shows that it has superior antimicrobial efficacy. In the Zone of Inhibition test, this formulation caused a 60 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 64 millimeter Zone of Inhibition for *Escherichia coli*. In the Plate Cidality test there was no growth observed for either of these organisms. Additionally, the Formulation exhibited Zones of Inhibition of 40 mm for *Candida albicans* and 52 mm for *Aspergillus niger*.

EXAMPLE 51

[0258] This example illustrates activity against spore-forming organisms.

[0259] Two difficult to kill spore forming microorganisms, *Bacillus anthracis* and *Bacillus subtilus* were challenged with the Formulation 14 in which one part of the Formulation 14 was diluted with two parts of water containing 20 ppm iron sulfate.

[0260] In the Zone of Inhibition test, a 79 mm zone of Inhibition was Observed with *Bacillus subtilus* and an 80 mm zone of inhibition with *Bacillus anthracis*. In the Plate Cidality test there was no growth observed for both of these microorganisms.

[0261] Formulation 14 was tested in a time to kill study with about an 8% spore population) bacteria. Approximately 1.0 ml of Formulation 14 were used in the study into which 10/8 cfu/ml was added and viable counts were measured over time intervals. The time intervals were 1, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12, 24 hours. Time intervals might be slightly different for different runs. At the designated time intervals about 1/1000 of an ml of solution was subcultured onto Petri dishes containing TSA (trypticase soy agar) with 5% Sheep Blood. The plates were incubated for about 24 hours and the number of colonies were counted if the counts were less than 300, otherwise counts were recorded as TNTC (too numerous to count). The results were recorded and plotted.

[0262] The results were as follows:

Time	Remaining Colonies
1 min	2
5 min	2
10 min	1
30 min	0
1 hour	1
2 hours	0
3 hours	0
4 hours	0
6 hours	0
12 hours	1
24 hours	1

[0263] Blank control Heavy growth, too numerous to count

EXAMPLE 52

[0264] The following is an example of an unstable peracetic acid containing antimicrobial formulation (Formulation 52).

[0265] Formulation 52 contained the following components:

Component	Mass (grams)
Water	11.63
cocobetaine - low salt version	21.4
Lauryl amine oxide	0.67
citric acid	0.2
Triclosan	1.1
Peracetic acid - 30%	15.0

[0266] Formulation 52 was prepared as follows:

[0267] A 250 ml beaker equipped with a magnetic stir bar and thermometer is charged with 11.63 grams of water, 21.4 grams of cocobetaine—low salt version, 0.67 grams of lauryl amine oxide, 0.2 grams of citric acid, The contents of the beaker are stirred and heated to about 60-63° C. At about 60° C. 1.10 grams of Triclosan are added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at about 60-63° C. for 20 minutes. The formulation is cooled to room temperature and 15.0 grams of 30% peracetic acid are added with stirring. The solution is stirred an additional 10 minutes at room temperature. The formulation has a pH of about 3.0. The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution.

[0268] This formulation began to degas almost immediately after preparation and after four months at room temperature continued to degas. Pressure in the storage bottle was relieved by loosening the cap on a weekly basis. Similar unstable formulations stored in plastic bottles which were not pressure relieved, bulged the plastic bottle considerably or ruptured the plastic bottles in less than two weeks at room temperature. This degassing of the formulation is an indication that the concentration of the active peroxy antimicrobial agent is constantly decreasing. Formulations with constantly changing concentrations of active ingredients have limited utility and applicability as an antimicrobial agent.

EXAMPLE 53

[0269] A peroxy formulation (Formulation 53) which is stable and efficacious contained the following components:

Component	Mass (grams)
Hydrogen peroxide 3% stabilized aqueous solution[1]	5.92
cocobetaine - low salt version	21.40
Lauryl amine oxide	0.67
citric acid	0.20
Triclosan	1.1
t-butyl hydroperoxide 70%	6.43
Peracetic acid - 30%	14.28

[0270] Aqueous hydrogen peroxide solutions are commonly stabilized with 25-250 ppm of colloidal stannate, sodium pyrophosphate, acetanilide or Dequest® organophosphates from Monsanto.

[0271] The total mass of the formulation is 50.0 grams and has a pH of about 3.0 and a % solids of about 15%.

[0272] The above formulation is diluted at room temperature by either of two ways before use:

[0273] a. 1 part of the above formulation with 2 parts of 3% aqueous hydrogen peroxide stabilized

[0274] b. 1 part of the above formulation with 2 parts of water containing 20 ppm iron sulfate

[0275] The formulation diluted as described for part a. above was tested for antimicrobial efficacy. Testing of this formulation shows that it has superior antimicrobial efficacy. In the Zone of Inhibition test, this formulation caused a 66 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 52 millimeter Zone of Inhibition for *Escherichia coli* and a 84 millimeter Zone of Inhibition for *Bacillus anthracis*.

[0276] In the Plate Cidality test there was no growth observed for any of these organisms.

[0277] Formulation 53 was prepared using the following procedure:

[0278] A 250 ml beaker equipped with a magnetic stir bar and thermometer is charged with 5.92 grams of 3% stabilized aqueous hydrogen peroxide, 21.40 grams of cocobetaine—low salt version, 0.67 grams of lauryl amine oxide, 0.2 grams of citric acid, The contents of the beaker are stirred and heated to about 60-63° C. The contents of the beaker were held at 60-63° C. for 10 minutes with stirring. After the 10 minute hold, at about 60° C. 1.10 grams of Triclosan are added with rapid stirring. In about 1-4 minutes a clear homogenous low viscosity solution forms. The solution is stirred at about 60-63° C. for 10 minutes. The formulation is cooled to room temperature and 6.43 g of 70% t-butyl hydroperoxide are added and stirred for about 1 minute to form a clear and homogeneous solution. Then 14.28 grams of 30% peracetic acid are added with stirring at

room temperature. The solution is stirred an additional 10 minutes at room temperature. The formulation has a pH of about 3.0. The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution. The above formulation and the two dilutions all were stable and showed no signs of degassing. After one month at room temperature none of the formulations showed any signs of degassing.

[0279] Formulations 54, 55 and 56 were prepared by the same procedure as in Formulation 53.

EXAMPLE 54

[0280] A peroxy formulation (Formulation 54) which is stable and efficacious contained the following components:

Component	Mass (grams)
Hydrogen peroxide 3% stabilized aqueous solution	5.92
cocobetaine - low salt version	34.18
Lauryl amine oxide	0.67
citric acid	0.20
Triclosan	1.10
t-butyl hydroperoxide 70%	6.43
Peracetic acid - 30%	1.50

[0281] The total mass of the above formulation is 50.0 grams and has a pH of about 4.5 and a % solids of about 23%.

[0282] The above formulation is diluted at room temperature by either of two ways before use:

[0283] a. 1 part of the above formulation with 2 parts of 3% aqueous hydrogen peroxide stabilized

[0284] b. 1 part of the above formulation with 2 parts of water containing 20 ppm iron sulfate

[0285] The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution. The above formulation and the two dilutions all were stable and showed no signs of degassing. After one month at room temperature none of the formulations showed any signs of degassing.

EXAMPLE 55

[0286] A peroxy formulation (Formulation 55) which is stable and efficacious contained the following components:

Component	Mass (grams)
Hydrogen peroxide 3% stabilized aqueous solution	5.92
cocobetaine - low salt version	21.40
Lauryl amine oxide	0.67
citric acid	0.20
Triclosan	1.10
t-butyl hydroperoxide 70%	6.43
Peracetic acid - 30%	5.00

[0287] The total mass of the above formulation is 40.72 grams and has a pH of about 3.5 and a % solids of about 15%.

[0288] The above formulation is diluted at room temperature as follows before use:

[0289] 1 part of the above formulation with 2 parts of 3% aqueous hydrogen peroxide stabilized

[0290] The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution. The above formulation and the dilution all were stable and showed no signs of degassing.

[0291] Testing of this formulation shows that it has superior antimicrobial efficacy. In the Zone of Inhibition test, this formulation caused a 80 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 101 millimeter Zone of Inhibition for *Escherichia coli* and a 100 millimeter Zone of Inhibition for *Bacillus anthracis*.

[0292] In the Plate Cidality test there was no growth observed for any of these organisms.

[0293] This formulation was tested in a time to kill study with about a 7% spore population bacteria. Approximately 1.0 ml of Formulation 14 were used in the study into which 10/8 cfu/ml was added and viable counts were measured over time intervals. The time intervals were 1, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12, 24 hours. Time intervals might be slightly different for different runs. At the designated time intervals about 1/1000 of an ml of solution was subcultured onto Petri dishes containing TSAR (triplicate soy agar) with 5% Sheep Blood. The plates were incubated for about 24 hours and the number of colonies were counted if the counts were less than 300, otherwise counts were recorded as TNTC (too numerous to count). The results were recorded.

[0294] The results were as follows:

Time	Remaining Colonies
1 min	0
5 min	0
10 min	0
30 min	0
1 hour	0
2 hours	0
3 hours	0
4 hours	0
6 hours	0
12 hours	0
24 hours	0

[0295] Blank control Heavy growth, too numerous to count

EXAMPLE 56

[0296] A peroxy formulation (Formulation 56) which is stable and efficacious contained the following components:

Component	Mass (grams)
Hydrogen peroxide 3% stabilized aqueous solution	10.20

-continued

Component	Mass (grams)
cocobetaine - low salt version	21.40
Lauryl amine oxide	0.67
citric acid	0.20
Triclosan	1.10
t-butyl hydroperoxide 70%	6.43
Peracetic acid - 30%	10.00

[0297] The total mass of the above formulation is 50.00 grams and has a pH of about 3.0 and a % solids of about 18%.

[0298] The above formulation is diluted at room temperature as follows before use:

[0299] 1 part of the above formulation with 2 parts of 3% aqueous hydrogen peroxide stabilized

[0300] The formulation is initially clear and homogeneous and remains clear and homogeneous upon sequential dilution. The above formulation and the dilution all were stable and showed no signs of degassing.

[0301] Testing of this formulation shows that it has superior antimicrobial efficacy. In the Zone of Inhibition test, this formulation caused a 88 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 88 millimeter Zone of Inhibition for *Escherichia coli* and a 150 millimeter Zone of Inhibition for *Bacillus anthracis*.

[0302] In the Plate Cidality test there was no growth observed for any of these organisms.

[0303] This formulation was tested in a time to kill study with about a 7% spore population bacteria. Approximately 1.0 ml of Formulation 14 were used in the study into which 10/8 cfu/ml was added and viable counts were measured over time intervals. The time intervals were 1, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12, 24 hours. Time intervals might be slightly different for different runs. At the designated time intervals about 1/1000 of an ml of solution was subcultured onto Petri dishes coptaining TSAR (triplicate soy agar) with 5% Sheep Blood. The plates were incubated for about 24 hours and the number of colonies were counted if the counts were less than 300, otherwise counts were recorded as TNTC (too numerous to count). The results were recorded.

[0304] The results were as follows:

Time	Remaining Colonies
1 min	0
5 min	0
10 min	0
30 min	0
1 hour	0
2 hours	0
3 hours	0
4 hours	0
6 hours	0
12 hours	0
24 hours	0

[0305] Blank control Heavy growth, too numerous to count

EXAMPLE 57

[0306] An all peroxy formulation (Formulation 57) with no Triclosan which is stable and efficacious contained the following components:

Component	Mass (grams)
Hydrogen peroxide 3% stabilized aqueous solution	3.15
cocobetaine - low salt version	10.70
Lauryl amine oxide	0.34
citric acid	0.10
t-butyl hydroperoxide 70%	3.22
Peracetic acid - 30%	7.50

[0307] The total mass of the above formulation is 25.00 grams and has a pH of about 3.0 and a % solids of about 12%.

[0308] The above formulation is diluted at room temperature as follows before use:

[0309] 1 part of the above formulation with 2 parts of 3% aqueous hydrogen peroxide stabilized

[0310] The above formulation and the dilution all were stable and showed no signs of degassing.

[0311] Testing of this formulation shows that it has superior antimicrobial efficacy. In the Zone of Inhibition test, this formulation caused a 62 millimeter Zone of Inhibition for *Staphylococcus aureus* and a 90 millimeter Zone of Inhibition for *Escherichia coli* and a 90 millimeter Zone of Inhibition for *Bacillus anthracis*.

[0312] In the Plate Cidality test there was no growth observed for any of these organisms.

[0313] Formulation 57 was prepared using the following procedure:

[0314] A 250 ml beaker equipped with a magnetic stir bar and thermometer is charged with 3.14 grams of 3% stabilized aqueous hydrogen peroxide, 10.70 grams of cocobetaine—low salt version, 0.34 grams of lauryl amine oxide, 0.1 grams of citric acid, The contents of the beaker are stirred and heated to about 60-63° C. The contents of the beaker were held at 60-63° C. for 15 minutes with stirring. The contents of the beaker are cooled to room temperature and some of the surfactant begins to gel or solidify. To the mixture are added at room temperature 3.21 g of 70% t-butyl hydroperoxide and stirred for about 15 minutes to form a clear and homogeneous solution. Then 7.50 grams of 30% peracetic acid are added with stirring at room temperature. The solution is stirred an additional 15 minutes at room temperature. The formulation has a pH of about 3.0. The formulation is stable and has no signs of degassing.

We claim:

1. An anti-microbial composition comprising:

at least one surfactant present in a concentration from about 3% to about 95% by weight;

an acid present in a concentration of up to about 20% by weight;

at least one non-cationic anti-microbial agent and the balance water,

wherein the amount of anti-microbial agent is effective to produce a Zone of Inhibition of from 15 to 150 mm and for the composition to exhibit a Plate Cidality of no growth against each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 at aqueous dilutions up to 1:2000 eliminate") and is CPE negative.

2. The antimicrobial composition of claim 1 wherein the Zone of Inhibition is in the range of from about 30 to 110.

3. The antimicrobial composition of claim 2 wherein the Zone of Inhibition is in the range of from about 45 to 75.

4. The antimicrobial composition of claim 1 which is effective to produce a Zone of Inhibition against *Staphylococcus aureus* ATCC 6538 of from 50 to 65.

5. The antimicrobial composition of claim 1 which is effective to produce a Zone of Inhibition against *Escherichia coli* ATCC 8739 of from 50 to 65.

6. The antimicrobial composition of claim 1 which is effective to produce a Zone of Inhibition against *Bacillus subtilus* and *Bacillus anthracis* of from 65 to 90 and a Plate Cidality of no growth against *Bacillus subtilus* and *Bacillus anthracis*.

7. The anti-microbial Formulation of claim 1, wherein said surfactant is selected from the group consisting of amphoteric surfactant, cationic surfactant, nonionic surfactant, anionic surfactant, and combinations thereof.

8. The anti-microbial composition of claim 1, wherein said compositions includes a first surfactant and a second surfactant.

9. The anti-microbial Formulation of claim 8, wherein said first surfactant is an amphoteric surfactant and said second surfactant is an anionic surfactant.

10. The anti-microbial composition of claim 1, wherein said surfactant is selected from the group consisting of alkylamine oxides, alkyamidopropyl amine oxides, alkyl betaines, alkyamidopropyl betaines, and sultaines.

11. The anti-microbial composition of claim 10, wherein said alkylamine oxide is selected from the group consisting of octyl amine oxide, decyl amine oxide, lauryl amine oxide, iso-dodecyl amine oxide, myristyl amine oxide, cetyl amine oxide, oleamine oxide, stearyl amine oxide, and palmitamine oxide.

12. The anti-microbial composition of claim 10, wherein said alkylamidopropyl amine oxide is selected from the group consisting of laurylamidopropyl amine oxide, cocamidopropyl amine oxide, stearamidopropyl amine oxide, germamidopropyl amine oxide.

13. The anti-microbial composition of claim 10, wherein said alkyl betaine is selected from the group consisting of octyl betaine, lauryl betaine, cocobetaine, cetyl betaine, oleyl betaine, and tallow dihydroxylethyl glycinate.

14. The anti-microbial composition of claim 10, wherein said alkylamidopropyl betaine is selected from the group consisting of caprylamidopropyl betaine, capramidopropyl betaine, lauamidopropyl betaine, cocamidopropyl betaine, isostearamidopropyl betaine, wheatgermaidopropyl betaine, and coco/sunfloweramidopropyl betaine.

15. The anti-microbial composition of claim 10, wherein said sultaine is selected from the group consisting of cocamidopropyl hydroxysultaine and lauryl hydroxysultaine.

16. The anti-microbial composition of claim 1, wherein said surfactant is selected from the group consisting of alkali metal salts of alkylamphoacetates, dialkali metal salts of alkylamphoacetates, alkali metal salts of alkylamphopropionates, dialkali metal salts of alkylamphopropionates, alkanolamides, disodium alkyl sulfosuccinates, monsodium diakyl sulfosuccinates, disodium alkylethoxy sulfosuccinates, sodium ethoxydimethicone sulfosuccinates, disodium propoxydimethicone sulfosuccinates, disodium alkylamido MEA sulfosuccinates, disodium alkylamido MPA sulfosuccinates, sodium alkylamidopropoxy sulfosuccinates, alkylamidopropylmorpholine lactate, and ethylene oxie/propylene oxide diblock and triblock surfactants.

17. The anti-microbial composition of claim 1, wherein said acid is an organic acid or an inorganic acid.

18. The anti-microbial composition of claim 17, wherein said organic acid is citric acid.

19. The anti-microbial composition of claim 17, wherein said inorganic acid is phosphoric acid.

20. The anti-microbial composition of claim 1, wherein said salt is an organic salt or an inorganic salt.

21. The anti-microbial composition of claim 20, wherein said organic salt is a citrate.

22. The anti-microbial composition of claim 20, wherein said inorganic salt is ammonium bifluoride.

23. The anti-microbial composition of claim 1, wherein said non-cationic antimicrobial agent is selected from the group consisting of phenolics, halogenated phenolics, hogenated diphenyl ethers, halogenated carbonilides, water soluble or water insoluble peroxy oxidizing agents, and combinations thereof.

24. The anti-microbial composition of claim 23, wherein said peroxy oxidizing agent is selected from the group consisting of peroxides, peresters, peracids, and persulfates.

25. The anti-microbial composition of claim 24, wherein said peroxy oxidizing agent is the group consisting of hydrogen peroxide, t-butyl hydroperoxide, cumene hydroperoxide and peracetic acid.

26. The anti-microbial composition of claim 1, wherein said non-cationic anti-microbial agent is Triclosan.

27. The anti-microbial composition of claim 7, wherein said amphoteric surfactant is cocobetaine.

28. The anti-microbial composition of claim 7, wherein said surfactant is an alkyl amine oxide.

29. The anti-microbial composition of claim 28, wherein said surfactant is lauryl amine oxide.

30. The antimicrobial composition of claim 7 wherein said anionic surfactant is a sulfosuccinate.

31. The anti-microbial composition of claim 1, wherein said anti-microbial composition possesses broad spectrum anti-microbial activity.

32. The anti-microbial composition of claim 31, wherein said broad spectrum anti-microbial activity includes antibacterial activity against Gram-negative bacteria and Gram-positive bacteria.

33. The anti-microbial composition of claim 1, wherein said anti-microbial composition is a clear and homogeneous composition.

34. A method of formulating an anti-microbial composition, comprising the steps of:

forming an admixture of water, and at least one surfactant;

heating said admixture to the melting point of a first solid non-cationic anti-microbial agent;

adding the non-cationic anti-microbial agent to said admixture while rapidly stirring at this melting point temperature; and

cooling said admixture to approximately room temperature,

35. The method of claim 34 wherein at least one acid is added to the admixture of water and surfactant.

36. The method of claim 34 wherein a second noncationic antimicrobial is added at room temperature after said cooling step.

37. The method of claim 34, further comprising the step of adding a peroxy oxidizing agent to said admixture after said cooling step.

38. A method of inhibiting the growth of microbes on a surface comprising the step of applying the composition of claim 1 to a surface.

39. A method of inhibiting the growth of microbes in a liquid, comprising the step of mixing the composition of claim 1 into a liquid in which microbial growth is desired to be inhibited.

40. A method of inhibiting the growth of microbes on the skin comprising applying the composition of claim 1 in combination with water to the skin.

41. A method of formulating an anti-microbial composition composed of:

at least one surfactant present in a concentration from about 5% to about 95% by weight;

at least one non-cationic anti-microbial agent present in a concentration of up to about 20% by weight; and

the balance water; comprising the steps of:

forming an admixture of said water, and said at least one surfactant;

heating said admixture to the melting point of a first non-cationic anti-microbial agent;

adding said non-cationic anti-microbial agent to said admixture while rapidly stirring at this melting point temperatures; and

cooling said admixture to approximately room temperature.

42. The method of claim 41 which further comprises adding at least one acid to the admixture in a concentration of up to about 20% by weight.

43. A method of formulating an anti-microbial composition composed of:

at least one surfactant present in a concentration from about 5% to about 95% by weight;

at least one non-cationic anti-microbial agent present in a concentration of up to about 20% by weight; and

the balance water; comprising the steps of:

mixing a solid antimicrobial agent with a liquid antimicrobial agent capable of dissolving or liquefying the solid antimicrobial agent, at ambient temperatures and agitating the mixture until a clear homogeneous Formulation is produced.

44. The method of claim 43 which further comprises adding at least one acid to the admixture in a concentration of up to about 20% by weight.

45. The method of claim 44 in which an aqueous solution of a peroxide is the liquid antimicrobial agent.

46. An antimicrobial composition obtained by the method of claim 43.

47. A method for killing microbes on a surface comprising applying antimicrobial cidal effective amount of the composition of claim 1 to the surface.

48. A method for rendering the atmosphere of an enclosed space antimicrobial comprising applying an antimicrobial effective amount of the composition of claim 1 to the atmosphere in the enclosed space.

49. An antimicrobial composition obtained by the method of claim 45.

50. An antimicrobial formulation comprising about 6.67 wt. % cocobetaine—low salt version, about 0.13 wt. % lauryl amine oxide, about 0.13 wt. % citric acid, about 0.73 wt, % triclosan, about 3.00 wt. % t-butyl hydroperoxide and about 13 ppm iron sulfate, the balance water.

51. An antimicrobial formulation comprising about 4% cocobetaine-low salt version, about 0.13% lauryl amine oxide, about 0.73% Triclosan, about 3% t-butyl hydroperoxide, about 2% peracetic acid and about 2% stabilized hydrogen peroxide, the balance water.

52. A method for preparing a stable peroxy antimicrobial formulation comprising adding from about 5 to 70% by weight of stabilized hydrogen peroxide solution to a surfactant solution, heating the mixture at approximately 60° C. for about 8 to 12 minutes, adding a noncationic antimicrobial agent with rapid stirring to obtain a clear homogenous low viscosity solution, stirring the solution at about 60-63° C. for 10 minutes, cooling the formulation to room temperature, adding an anti-microbial peroxide component and stirring the mixture for one to five minutes to form a clear and homogeneous solution which does not degas.

53. The method of claim 52 wherein the amount of stabilized hydrogen peroxide added is between 15 and 50% by weight of stabilized hydrogen peroxide.

54. The method of claim 52 wherein the resulting formulation is diluted with a solution of stabilized hydrogen peroxide solution, an aqueous solution of iron sulfate or water.

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